

## EVALUATION OF A RAPID TEST TO DETECT CARBAPENEMASE PRODUCTION AMONG GRAM-NEGATIVE BACILLI

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### ABSTRACT

**Objective:** There is a need for an early detection of carbapenemase-producing Gram-negative bacilli to reduce the morbidity and mortality associated with it. The objective of this study is to evaluate the usefulness of the Carba NP test in detecting carbapenemase-producing Gram-negative bacilli.

**Methods:** This prospective experimental study was conducted in 2013 at PSG Institute of Medical Sciences and Research, Coimbatore. In this study, 120 consecutive Gram-negative bacilli clinical isolates identified as carbapenem resistant as per the Clinical and Laboratory Standard Institute guidelines were subjected to Carba NP.

**Results:** About 62.5% of the carbapenem-resistant isolates were found to be carbapenemase producers by modified Hodge test. The sensitivity of the Carba NP test was found to be 92% and the specificity was 100%. The most common bacterial isolate was *Acinetobacter baumannii*, and the most common sample was urine.

**Conclusion:** The Carba NP test is a simple inexpensive, rapid, reproducible, sensitive test that can be performed directly from the colonies. It improves early detection of patients infected or colonized with carbapenemase in the hospital.

**Keywords:** Carba NP test, Carbapenemase producer, Modified Hodge test.

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### INTRODUCTION

Carbapenem-resistant Gram-negative bacilli have emerged and are being reported globally at increasing rates. Carbapenem sensitivity of *Pseudomonas aeruginosa* strains isolated from patients with urinary tract infection was only 37% in a study conducted in South India [1]. With limited treatment options and the high morbidity and mortality associated with infections caused by them, it is a huge concern requiring immediate scientific attention. Carbapenem resistance due to non-carbapenemase-mediated mechanisms includes outer membrane permeability defect, efflux mechanisms, loss of porins associated with overproduction of cephalosporinase, and/or extended-spectrum  $\beta$ -lactamases or from strains that are carbapenem susceptible but express a broad-spectrum  $\beta$ -lactamase without carbapenemase activity, extended-spectrum  $\beta$ -lactamase, and plasmid- and chromosome-encoded cephalosporinases [2]. Carbapenemase production is coded on plasmids and transferred from one bacterium to the other very easily. There is a need for an early detection of carbapenemase-producing Gram-negative bacilli and isolate patients harboring them and treat them effectively. The currently used Clinical and Laboratory Standard Institute (CLSI) recommended modified Hodge test (MHT) for the detection of carbapenemase takes 18-24 h [3]. To detect carbapenemase production, Carba NP test was developed by Nordmann *et al.* based on the principle of hydrolysis of carbapenem [4].

### MATERIALS AND METHODS

#### Materials

After obtaining an Institutional Human Ethics Committee approval, this prospective experimental study was conducted at PSG Institute of Medical Sciences and Research, Coimbatore, over a period of 6 months in 2013 on 120 consecutive Gram-negative isolates identified as carbapenem-resistant by MHT as per the CLSI-2013 guidelines out of 2590 Gram-negative bacilli isolates obtained from a range of samples which included urine, pus, blood, sputum, tracheal aspirate, catheter

tip, tissue, and body fluids. Carbapenemase production of these isolates was detected by subjecting them to MHT and Carba NP test.

#### Methods

##### MHT

A 0.5 McFarland dilution of the *Escherichia coli* ATCC 25922 in 5 ml of Mueller-Hinton (MH) broth was prepared. Lawn culture was made on the MH agar plate with 1:10 dilution of this solution, and a meropenem disc (30  $\mu$ g) was placed in the center. In a straight line, test organism was streaked from the edge of the disk to the edge of the plate and was incubated at 37°C for 16–24 h. Clover leaf-like indentation of the *E. coli* 25922 along the streak of the test organism growth within the diffusion zone of the disk was identified as positive [3].

##### Carba NP test

This test was performed on strains grown on MH agar plates at 37°C for 18–22 h. One calibrated loop (10  $\mu$ l) of the tested strain directly obtained from the MH agar plate was re-suspended in 100  $\mu$ l of a 20 mM Tris-HCl lysis buffer vortexed for 1 min and was further incubated at room temperature for 30 min. This bacterial suspension was then centrifuged at 10,000 rpm at room temperature for 5 min. Thirty microliters of the supernatant was mixed in a well of a 96-well tray with 100  $\mu$ l of imipenem monohydrate (3 mg/ml) solution, phenol red solution, and 0.1 mM ZnSO<sub>4</sub> and was incubated at 37°C for a maximum of 2 h. Change in color from red to orange or yellow was interpreted as positive and no change in color as negative [4].

#### RESULTS

In our study, 120 (4.63%) isolates were found to be carbapenem resistant, of the 2590 Gram-negative bacilli isolated from various clinical samples. Urine was the predominant sample 47% (56), followed by respiratory samples (27), pus sample (26), blood (8), and others (3). Of the 120 samples included in the study, *Acinetobacter baumannii*

was the predominant organism 47.5% (57), followed by *Klebsiella pneumoniae* (29), *P. aeruginosa* (21), *E. coli* (8), *Enterobacter* sp. (3), *Proteus* sp. (1), and *Citrobacter* sp. (1) as mentioned in Table 1.

We found that 62.5% (75) of the isolates were positive by MHT. The sensitivity of the Carba NP test was found to be 92% (69), and the specificity was 100% (Table 2). The positive predictive value of Carba NP test was 100%, and the negative predictive value was 88.24%.

## DISCUSSION

Carbapenems, the last-line drug of therapy, are increasingly reported to be resistant to Gram-negative bacilli causing severe infections. Early detection of these organisms is important to initiate appropriate therapy and for the implementation of infection control practices. In our study, we found 62.5% of the carbapenem-resistant organism as carbapenemase producers by MHT. Other isolates might be carbapenem resistance due to non-carbapenemase-mediated mechanisms. Among many mechanisms of carbapenem resistance, carbapenemase production is the most important one.

The sensitivity and specificity of the Carba NP test were described to be 100% by Dortet Poirel and Nordmann *et al.* However, in our study, we found the sensitivity to be low (92%). Similar to results in our study, evaluation of Rapidec Carba NP test, a commercially available test for detection of carbapenemases in Enterobacteriaceae derived from the Carba NP-I assay, had sensitivity and specificity of 90.2% and 100%, respectively, when strictly followed the manufacturer's instructions [5]. In our study, the type of carbapenemase produced was not studied. Carba NP test II developed by Dortet *et al.* enabled to identify carbapenemase production in Gram-negative bacilli and also to discriminate between the different types of carbapenemases. It is based on the detection of the acidification resulting from imipenem hydrolysis, coupled with tazobactam and ethylenediaminetetraacetic acid (EDTA) as inhibitors [6]. In our study, 3 mg/ml concentration of imipenem and supernatant obtained after centrifuging the lysed bacterial cells was used for the final reaction. Vasoo *et al.* utilized whole bacterial cells instead of supernatant obtained from bacterial lysis and an increased concentration of imipenem (6 instead of 3 mg/ml) and found that their approach enabled detection of carbapenemase-producing *Pseudomonas* species isolates which were initially negative

when tested with 3 mg/ml of imipenem and supernatant obtained after bacterial lysis [7]. Apart from the use of supernatant and an increased concentration of imipenem, sensitivity of the test can be affected by extraction process, expression of gene, hydrolyzing ability of the carbapenemase, stability of carbapenem, inhibitors present, and the pH of the solution. Of 36 isolates of carbapenemase-producing *A. baumannii*, only 33 isolates were detected as carbapenemase producers by Carba NP test in our study. A modified version of Carba NP test and CarbAcineto test, was developed by Dortet *et al.* to enhance the detection rates of carbapenemase-producing *Acinetobacter* species by replacing the lysis buffer by a 5 M NaCl solution, avoiding any buffer effect, and doubling the bacterial inoculum from one-third to one-half of a calibrated loop (10 µl) to a whole calibrated loop to increase the enzyme quantity [8].

The huge advantage of Carba NP test is that the interpretable positive results were obtained in <2 h, making it possible to implement rapid containment measures to limit the spread of carbapenemase producers. Carba NP test may be useful in particular for intensive care unit and burns patients, among whom multidrug-resistant isolates are widespread. It offers a cost-effective solution for detecting carbapenemase-producing Gram-negative bacilli and preventing their spread, considering that they may harbor those carbapenemase producing genes on plasmids that can spread to other bacterial families (Enterobacteriaceae and the family that includes *Acinetobacter* species). The limitation of the study is that molecular diagnosis of carbapenemase producer and typing of carbapenemases were not included in the study. Combination therapy is recommended for carbapenem-resistant Gram-negative organisms. However, colistin-carbapenem combination therapy has been observed to have low clinical success rate probably due to indifferent interactions between them [9].

## CONCLUSION

Including this sensitive and rapid test in routine evaluation of the bacterial isolates can initiate prompt treatment and aggressive infection control measures to prevent its spread in the health-care facility.

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**Table 1: Results of carbapenemase detection by MHT and Carba NP test**

Organisms	Number of samples tested	MHT test +VE	Carba NP test +VE
<i>A. baumannii</i>	57	36	33
<i>K. pneumoniae</i>	29	19	18
<i>P. aeruginosa</i>	21	12	11
<i>E. coli</i>	8	5	5
<i>Enterobacter</i> sp.	3	2	1
<i>Proteus</i> sp.	1	1	1
<i>Citrobacter</i> sp.	1	-	-
Total	120	75	69

*A. baumannii*: *Acinetobacter baumannii*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *E. coli*: *Escherichia coli*, MHT: Modified Hodge test

**Table 2: Comparison of MHT and Carba NP test in detecting carbapenemases**

	Carba NP test (+)	Carba NP test (-)	Total
MHT (+)	69	6	75
MHT (-)	0	45	45
Total	69	51	120

MHT: Modified Hodge test